Effect of carbetocin, oxytocin and prostaglandin E2 and F2alpha on intrauterine pressure in cows in dioestrus and oestrus

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Abstract: OBJECTIVE: To describe the physiological activity of the myometrium in oestrus and dioestrus and the induced activity after medication in cows with particular reference to segmental differences. MATERIAL AND METHODS: Six cows were given the pharmaceuticals carbetocin, oxytocin and prostaglandin (PG) F2 (dinoprost) intramuscularly and PGE2 intravenously. The physiological myometrial activity was recorded for 15 minutes and the induced activity for 105 minutes by using a transcervically attached pressure probe containing six pressure microtransducers. RESULTS: Lower pressures were measured in dioestrus compared to oestrus before (dioestrus 3.2 ± 8.88 mmHg, oestrus 12.4 ± 13.23 mmHg, $p < 0.0001$) and after the drug administration. Carbetocin provoked the longest lasting effect (60 minutes in dioestrus, 75 minutes in oestrus) followed by PGE2 (45 minutes in dioestrus, 60 minutes in oestrus), PGF2 (30 minutes each) and oxytocin (15 minutes in oestrus only). In contrast to the other drugs carbetocin did not cause any pressure decrease beneath the base level after the primary pressure increase in dioestrus. In dioestrus the pressure before drug administration was significantly higher in the cervix (3.6 ± 19.40 mmHg) and the uterine body (7.1 ± 36.10 mmHg) than in the uterine horn (1.1 ± 7.21 mmHg). Conversely, in oestrus the pressure in the uterine horn (16.6 ± 17.73 mmHg) was significantly higher than in the uterine body (6.2 ± 16.59 mmHg) and the cervix (10.4 ± 17.91 mmHg). Drug administration in dioestrus caused a cornual pressure increase and the pressure in the uterine body decreased, whereas in oestrus the pressure increased in all uterine segments. The physiological frequency of the pressure waves in dioestrus was 5.2 ± 3.02 in 15 minutes compared to 7.5 ± 2.89 in 15 minutes in oestrus. No traceable changes of the contraction frequency were found after medication. CONCLUSION AND CLINICAL RELEVANCE: Carbetocin caused the most enduring increase in intrauterine pressure in dioestrus and oestrus and may therefore be indicated best for therapeutic use. The tested drugs had the same effects on the various uterine segments and no effect on the contraction frequency.

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Effect of carbetocin, oxytocin and prostaglandin E_2 and $F_{2\alpha}$ on intrauterine pressure in cows in dioestrus and oestrus

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Keywords

Cattle, uterus, intrauterine pressure, carbetocin, oxytocin, prostaglandin E_2, prostaglandin $F_{2\alpha}$

Summary

Objective: The aim of this study was to describe the physiological activity of the myometrium in oestrus and dioestrus and the induced activity after medication in cows with particular reference to segmental differences. **Material and methods:** Six cows were given the pharmaceuticals carbetocin, oxytocin and prostaglandin $F_{2\alpha}$ (dinoprost) intramuscularly and prostaglandin E_2 intravenously. The physiological myometrial activity was recorded for 15 minutes and the induced activity for 105 minutes by using a transcervically attached pressure probe containing six pressure microtransducers. **Results:** Lower pressures were measured in dioestrus compared to oestrus before (dioestrus 3.2 ± 8.88 mmHg, oestrus 12.4 ± 13.23 mmHg, $p < 0.0001$) and after the drug administration. Carbetocin provoked the longest lasting effect (60 minutes in dioestrus, 75 minutes in oestrus) followed by prostaglandin E_2 (45 minutes in dioestrus, 60 minutes in oestrus), prostaglandin $F_{2\alpha}$ (30 minutes each) and oxytocin (15 minutes in oestrus only). In contrast to the other drugs carbetocin did not cause any pressure decrease beneath the base level after the primary pressure increase in dioestrus. In dioestrus the pressure before drug administration was significantly higher in the cervix (3.6 ± 19.40 mmHg) and the uterine body (7.1 ± 36.10 mmHg) than in the uterine horn (1.1 ± 7.21 mmHg). Conversely, in oestrus the pressure in the uterine horn (16.6 ± 17.73 mmHg) was significantly higher than in the uterine body (6.2 ± 16.59 mmHg) and the cervix (10.4 ± 17.91 mmHg). Drug administration in dioestrus caused a cornual pressure increase and the pressure in the uterine body decreased, whereas in oestrus the pressure increased in all uterine segments. The physiological frequency of the pressure waves in dioestrus was 5.2 ± 3.02 in 15 minutes compared to 7.5 ± 2.89 in 15 minutes in oestrus. No traceable changes of the contraction frequency were found after medication. **Conclusion and clinical relevance:** Carbetocin caused the most enduring increase in intrauterine pressure in dioestrus and oestrus and may therefore be indicated best for therapeutic use. The tested drugs had the same effects on the various uterine segments and no effect on the contraction frequency.

Schlüsselwörter

Rinder, Uterus, intrauteriner Druck, Carbetocin, Oxytocin, Prostaglandin E_2, Prostaglandin $F_{2\alpha}$

Zusammenfassung

Gegenstand und Ziel: Untersuchung der physiologischen und induzierten myometralen Aktivität nach Medikamentengabe bei Kühen unter besonderer Berücksichtigung segmentaler Unterschiede in den Zyklusstadien Östrus und Diöstrus. **Material und Methoden:** Sechs Kühen wurden die Wirkstoffe Carbetocin, Oxytocin und Prostaglandin $F_{2\alpha}$ (Dinoprost) intramuskulär sowie Prostaglandin E_2 intravenös appliziert. Mittels einer transzervikal eingebrachten Druckmesssonde mit sechs Messpunkten wurde die physiologische Motorik über 15 Minuten und die induzierte Motorik ber 105 Minuten aufgezeichnet. **Ergebnisse:** Im Diöstrus ergaben sich sowohl vor als auch nach Medikamentengabe in allen Fällen niedrigere Drücke als im Östrus (Diöstrus 3,2 ± 8,88 mmHg, Östrus 12,4 ± 13,23 mmHg, $p < 0,0001$).

Carbetocin zeigte die am längsten messbare Wirkung (60 Minuten im Diöstrus, 75 Minuten im Östrus), gefolgt von Prostaglandin E_2 (45 Minuten im Diöstrus, 60 Minuten im Östrus), Prostaglandin $F_{2\alpha}$ (je 30 Minuten) und Oxytocin (15 Minuten nur im Östrus). Zudem bewirkte Carbetocin im Diöstrus im Gegensatz zu den anderen Wirkstoffen nach dem primären Druckanstieg keinen Druckabfall unter das Ausgangsniveau. Vor der Medikation war der Druck im Diöstrus in der Zervix (3,6 ± 19,40 mmHg) und im Uteruskörper (7,1 ± 36,10 mmHg) signifikant höher als im Uterushorn (1,1 ± 7,21 mmHg). Im Östrus hingegen lag der Druck im Uterushorn (16,6 ± 17,73 mmHg) signifikant höher als im Uteruskörper (6,2 ± 16,59 mmHg) und in der Zervix (10,4 ± 17,91 mmHg). Die Medikamentengabe bewirkte im Diöstrus einen Druckanstieg in den Uterushörnern und einen Druckabfall im Uteruskörper, während im Östrus der Druck in allen Segmenten anstieg. Die physiologische Frequenz der Kontraktionswellen war im Diöstrus mit 5,2 ± 3,02 in 15 Minuten niedriger als im Östrus mit 7,5 ± 2,89 in 15 Minuten. Nach der Medikation kam es zu keiner nachvollziehbaren Änderung der Kontraktionsfrequenz. **Schlussfolgerung und klinische Relevanz:** Carbetocin bewirkte in beiden Zyklusstadien den am längsten anhaltenden intrauterinen Druckanstieg und scheint daher am geeignetsten für eine therapeutische Anwendung. Die verwendeten Uterotonika unterschieden sich nicht in ihrer Wirkung auf die verschiedenen Uterussegmente und hatten keinen Einfluss auf die Kontraktionsfrequenz.

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Uterusdruckmessungen beim Rind unter Einfluss von Carbetocin, Oxytocin sowie Prostaglandin $_{E2}$ und $F_{2\alpha}$

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Introduction

The coordinated muscle activity of the bovine uterus has several important functions in reproductive events during oestrus, pregnancy, parturition and the puerperal period when the placenta, lochia or pathological uterine contents are expelled. In general terms, the uterine body and horns have a transport function and the uterine cervix serves as a barrier. Several studies evaluated the effect of various uterotonic drugs in relation to these functions because of considerable interest in their therapeutic use (2, 4, 6, 9, 14–17, 19, 27, 29, 30, 34). Microtransducers represent the most advanced method to measure intrauterine pressure in veterinary medicine. The well-established technique has been used in former studies using one (34), two (28–30) or three (15–17, 19) microtransducers in a pressure probe.

Oxytocin and its synthetic analogue carbetocin increased the frequency of uterine contractions for 2 hours and the total area under the curve for intrauterine pressure for 1 hour in cows (6). Oxytocin, a peptide hormone, is produced in the hypothalamus and secreted by the pituitary gland. It is also produced and secreted by the corpus luteum. In circulation it has a half-life of 1–8 minutes. Oxytocin causes the smooth musculature of the female genital tract and mammary gland to contract (5). The uterine effect of the hormone is a function of the oxytocin receptor concentration in endometrium and myometrium having its peak in oestrus and its lowest concentration around day 9 of the cycle (5, 13, 22). These differences were also

described in the cervix (13). Carbetocin has the same general properties but because of its greater stability its half-life is much longer with up to 6 hours (5).

Another study investigated the uterotonic effects of different prostaglandins in cattle (19). Prostaglandin E_2 (PGE_2) is an inflammatory mediator, protects the gastric mucosa and controls reproductive events. It is of ovarian and endometrial origin and together with other factors is involved in the process of ovulation. It also causes an increase in intracellular calcium concentration and thus activates uterine muscle contractions (5, 37). Prostaglandin E_2 enhanced uterine motility for up to 45 minutes in cattle and shortened the duration of parturition (17, 19, 21, 29). A PGE_2 "relaxant" receptor (EP2) was described, which is expressed in endometrium and myometrium during the estrous cycle with a peak between day 10 and day 18 (3). EP2 is upregulated in early pregnancy, presumably to induce uterine quiescence. This was also shown in late pregnany and parturition, indicating that an upregulation of contractile factors overcomes the inhibitory effects of EP2 at parturition (35). In women, PGE_2 causes uterine contraction and cervical relaxation (10, 31).

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), another inflammatory mediator, is synthesised in the endometrium towards the end of the luteal phase and causes luteolysis. It is responsible for increased uterine motility via an increase in intracellular calcium concentration. The first plasma metabolite of $PGF_{2\alpha}$ is 15-keto-13,14-dihydro-$PGF_{2\alpha}$, which has a half-life of 3–8 minutes (5). The effect of $PGF_{2\alpha}$ and its analogues on the myometrium generally consists of increased muscular activity; however, this effect does not occur with all analogues and not at every cycle stage (17, 19, 27, 29, 34). This led to the conclusion that the stimulating effect of $PGF_{2\alpha}$ on the uterine muscle is contingent on the type of analogue but also on the stage of the oestrous cycle (34). The latter might be a result of changes in the concentration of the "contractile" $PGF_{2\alpha}$ receptor (FP) during the oestrous cycle. It has been shown, that the amount of FP is rising from late term gestation to parturition (35). In addition to its direct uterotonic effect, the ability of $PGF_{2\alpha}$ to cause luteolysis (5) results in a greater intrauterine pressure during the following oestrus (2, 9, 18, 27, 28, 32) facilitating the expulsion of uterine content. This represents a secondary therapeutic effect, e. g. in case of endometritis.

The hypothesis of this study was that oxytocin, carbetocin, PGE_2 and $PGF_{2\alpha}$ cause an increase in uterine tone that can be quantified in different segments of the uterus. Qualitative and quantitative differences between the effects of the tested drugs should provide an indication of the therapeutic usefulness, e. g. the evacuation of the uterus in case of an endometritis. Therefore the goal was to measure the strength and duration of intrauterine pressure before and after treatment with uterotonic drugs using a microchip precision pressure catheter with six semi-conductor pressure sensors. This kind of catheter, which has never before been used in a similar setting, makes it possible to measure the pressure in different uterine segments simultaneously and thereby comparable. A secondary goal was to investigate physiological differences in pressure between different uterine segments during oestrus and dioestrus**.**

Material and methods

Animals

Six multiparous Swiss Braunvieh cows that were at least 8 weeks in milk and between 5 and 16 years old from a university-owned research farm were used. The cows had to be assessed healthy in the clinical and gynaecological examinations before they were included in the experiment.

Pressure catheter and fixation

The pressure catheter was a Gastrobar® microchip precision pressure catheter with six semi-conductor pressure sensors (microtransducers) custom-made for this study (Raumedic, Münchberg, Germany). The probe had an average diameter of 3.5 mm and the first pressure

sensor (P1) was 2 cm from the tip of the probe (Fig. 1). The remaining pressure sensors (P2 to P6) were located at 5-cm intervals so that the sixth sensor was 27 cm from the tip of the probe.

The microchip precision pressure catheter was introduced into a uterine horn via a sheath (modified Detlef biopsy instrument reaching from the handle to the external cervical os; Fig. 1). After placement of the pressure catheter (A, green catheter visible in the opened uterine horn and at the caudal end of the sheath) a piece of plastic tubing (D, custom-made from the sheath of a swabbing instrument) with the same length as the sheath and with a slit along its entire length was clipped onto the catheter piece by piece and slid towards the cervix. The caudal end of the tubing rested against the two rings at the caudal end of the sheath, which was attached to the perineal tissue with an elastic band (not shown in Fig. 1). The tubing served to fix the pressure catheter in place. A 20-mm bulb attached to the tip of the stealth was lodged against the external cervical os by force of the elastic perineal attachment and kept the tube and catheter from entering the cervix. The premeasured position of the 77-cm mark of the pressure catheter at the caudal end of the catheter ensured that the most caudally located measuring point P6 was located 4 cm cranial to the bulb-end of the plastic tubing in the cervix. The pressure data were transmitted to an attached measuring unit (Ellipse, Andromeda, Taufkirchen/Potzham, Germany) and from there to a laptop. The software AUDACT (Andromeda) was used to store and analyse the data.

Uterotonic drugs

Experimental drugs included carbetocin (LongActon®, Vital, Switzerland), oxytocin (Physovetin®, Streuli Pharma, Switzerland), prostaglandin E_2 (Dinoproston, Myoton E_2®, Graeub, Switzerland) and prostaglandin $F_{2\alpha}$ (Dinoprost, Dinolytic®, Zoetis, Switzerland). The dosages and routes of administration were chosen according to the manufacturers' recommendations and were as follows: 30 IU for oxytocin, intramuscularly (i. m.); 0.35 mg for carbetocin, i. m.; 25 mg for prostaglandin $F_{2\alpha}$, i. m.; and 2.5 mg for prostaglandin E_2 2.5 mg, intravenously into a jugular vein. The triceps muscle of the forearm was used for intramuscular injections.

Determination of cycle stage

To determine the cycle stage, the ovaries of the cows were examined transrectally once daily using B-mode sonography until ovulation was detected, which was defined as the first day that a follicle was no longer visible on the sonograms (day 0). After ovulation, the daily examinations were continued to verify the cycle stage.

A corpus luteum with a diameter ≥ 23 mm combined with a plasma progesterone concentration > 1 ng/ml was defined as dioestrus (7, 8). A corpus luteum with a diameter < 23 mm combined with a plasma progesterone concentration ≤ 1 ng/ml and one or several follicles with a diameter ≥ 12 mm was defined as oestrus.

For determination of plasma progesterone concentration, 10 ml blood was collected from a jugular vein into a lithium heparin tube (Sarstedt, Nümbrecht, Germany) and centrifuged. The harvested plasma was stored at –18 °C until analysis. Progesterone was measured at the Endocrinology Laboratory at the Clinic for Obstetrics, Gynaecology and Andrology for Large and Small Animals and Ambulatory Field Service, Justus-Liebig-University, Giessen, Germany using a radioimmunoassay (24).

Study design

Each of the six cows underwent one pressure measurement with each of the four experimental drugs during oestrus and during dioestrus for a total of 48 measurements. Oxytocin or carbetocin were tested on day 10 and PGE_2 or $PGF_{2\alpha}$ after a wash-out period of 48 hours (day 12) of the same cycle. Oxytocin or carbetocin was then tested on the first day of the next oestrus, and PGE_2 or $PGF_{2\alpha}$ after a wash-out period of 24 hours to ensure that both measurements were carried out during the same oestrus. The same course of measurements

was done starting with the second peptide hormone (oxytocin or carbetocin) and the second prostaglandin (PGE_2 or $PGF_{2\alpha}$).

Pressure measurement procedure

The pressure catheter was calibrated to barometric pressure and set to 0 before intrauterine placement. The cows were allowed 20 minutes to adapt to the catheter. At the beginning of every measurement (four in dioestrus and four in oestrus per cow), recordings of the physiological uterine pressure were carried out for 15 minutes (baseline measurement). The test drug was then given and the pressure recorded for another 105 minutes. Measuring artefacts defined as impacts on the pressure sensors that were not related to changes in myometrial tone such as urination, defecation, coughing, vocalisation or straining to expel air from the vagina were noted.

Side effects

Heart and respiratory rates and possible side effects were recorded before the start of the experiment, 10 minutes after the start of pressure recordings and 5, 30, 60 and 120 minutes after administration of the test drug.

Data processing

Each measuring point of the pressure catheter yielded data sets with 20 pressure readings per second. These data sets were transferred to an Excel spread sheet (Microsoft, Wallisellen, Switzerland) and means were calculated for a 15-minute period (minutes 15–29, referred to as baseline) before and seven 15-minute periods (minutes 31–45, 46–60, 61–75 etc.) after drug administration. The readings from the six measuring points were used for comparison of the pressure in different uterine segments, and the means of all six measuring points were used for comparison of the overall pressure. In case of artefacts, a software program (AUDACT, Andromeda) was used to delimit and eliminate the affected recordings, and the means were calculated for the remaining data of the respective 15-minute period.

Endpoints

Oestrous cycle-related changes in overall uterine pressure were analysed using the means of the eight time periods during oestrus and dioestrus for all drugs combined.

Differences in pressure between individual measuring points were analysed for all eight time periods during dioestrus and oestrus for all drugs combined. Differences in pressure between individual measuring points were analysed during oestrus and dioestrus for each drug by comparing time periods 1 and 3. Time period 3 (i. e., the second after medication) was associated with the greatest pressure increase for all pressure points.

The duration of effect of each drug was determined during dioestrus and oestrus by comparing the mean overall pressure during time period 1 with the pressures during time periods 2 to 8. This allowed determination of the duration of effect for each drug with an accuracy of 15 minutes.

Statistics

The mean uterine pressures recorded at the eight measuring periods were analysed using StatView Version 5.0 (SAS Institute, Cary, USA). The Shapiro-Wilk test was employed to test the data for normal distribution. Because all data were normally distributed, they were reported as mean ± standard deviation. Including the individual differences in the statistical models differences in pressure between time periods were analysed for both cycle stages separately or combined for each drug using a paired t-test.

Student's t-test for paired samples was used to analyse differences in mean overall pressure at all time periods between oestrus and dioestrus for each drug separately and for all drugs combined, to analyse differences in mean pressure at the individual measuring points and in

each time period for all drugs and both cycle stages combined and to analyse differences in mean pressure recorded at the different measuring points between time periods 1 and 3. A p-value ≤ 0.05 was considered significant.

This study was approved by the Committee for the Permission of Animal Experimentation of the Canton of Zurich (04/2007).

Results

Plasma progesterone concentration

The mean progesterone concentration of the cows diagnosed sonographically as oestrous was 0.59 ± 1.13 ng/ml. With one exception the plasma progesterone concentrations were < 1 ng/ml and were in agreement with clinical and sonographic findings. The exception was a cow with a progesterone concentration > 1 ng/ml in a repeated assay, accompanied by a corpus luteum measuring 22 mm in diameter and a dominant follicle that was no longer visible the next day. The measurements on this test day were therefore included for calculation of the mean for the cows in oestrus.

In two cows, no blood was collected when they were tested in oestrus. The mean progesterone concentration of the cows diagnosed clinically and sonographically as dioestrous was 5.45 ± 1.34 ng/ml.

Side effects

Adverse signs such as colic were not seen, which indicated that the wellbeing of the cows was not affected by the measuring equipment. Some cows reacted with defensive movements during drug administration and insertion of the pressure catheter, but these reactions subsided during the adaption period. Three of the six cows were dripping milk a few minutes after oxytocin and carbetocin administration during oestrus and dioestrus.

Pressure differences

Cycle stage-related pressure differences

The mean overall pressure was greater during oestrus than during dioestrus in each time period for all drugs combined (all $p < 0.001$, Fig. 2). The absolute differences in pressure before and after administration of the 4 drugs were significantly greater during oestrus than during dioestrus (all $p < 0.001$).

Segmental pressure differences before medication

Intrauterine pressures for all drugs combined in the six uterine segments before and after medication are shown in Table 1. In dioestrus, the mean pressure ranged from 0.9 ± 8.63 mmHg at P4 to 7.1 ± 36.10 mmHg at P5 (Fig. 3). Pressures at P2, P5 and P6 were significantly greater than at the other measuring points except for P6 and P1, which were the same. In oestrus, the mean pressure ranged from 6.2 ± 16.59 mmHg at P5 to 18.5 ± 17.12 mmHg at P2 (Fig. 4). Pressures at P2 and P3 were significantly greater than at the other measuring points.

Segmental pressure differences after medication for all drugs combined

In dioestrus, intrauterine pressure increased at P1 to P4 and decreased at P5 and P6 causing an intrauterine pressure gradient. With one exception, pressures at P1 to P4 were greater than those at P5 and P6 (Fig. 3). In oestrus, intrauterine pressure increased at all measuring points and the intrauterine pressure differences remained largely uniform; pressure at P2 and P3 was significantly greater than at the other measuring points. Pressures at P5 and P6 were uniform and, with three exceptions out of 12 measurements, significantly lower than at P1 and P4, which were in the intermediate pressure range (Fig. 4).

The time periods 1 and 3 were compared to analyse the effect of medication on intrauterine pressure. In dioestrus, the pressure at P1 to P4 increased significantly after medication (all

$p < 0.0001$) and at P5 it decreased ($p < 0.01$). There was a numerical decrease at P6 ($p = 0.068$, Fig. 5). In oestrus, the pressures at all measuring points increased significantly (Fig. 6). The greatest increase from 16.6 ± 17.73 mmHg to 27.6 ± 26.22 mmHg occurred at P3 ($p < 0.0001$) and the smallest from 10.4 ± 17.91 mmHg to 12.5 ± 18.42 mmHg at P6 ($p < 0.05$).

Segmental pressure differences after medication with individual drugs

In dioestrus, **carbetocin** caused an increase in intrauterine pressure at P1 to P4 (all $p < 0.0001$) but not at P5 and P6. In oestrus, it caused an increase at P1 to P5 (P1 $p < 0.05$; P2–P5 $p < 0.0001$) but not at P6.

In dioestrus, **oxytocin** caused an increase in intrauterine pressure at P1 to P4 (P1 $p < 0.001$; P2 $p \leq 0.05$; P3 and P4 $p < 0.0001$) and a decrease in pressure from 14.5 ± 37.75 mmHg to –2.1 ± 12.64 mmHg at P5 ($p < 0.001$) and from 6.0 ± 28.24 mmHg to –2.9 ± 8.79 mmHg at P6 ($p < 0.01$). In oestrus, it caused an increase at P2 ($p < 0.05$) and P3 ($p < 0.001$).

In dioestrus, **prostaglandin E_2** caused an increase in intrauterine pressure at P1, P3, P4 and P5 (all $p < 0.0001$). In oestrus, it caused an increase at P1 to P5 (P1, P3, P4 $p < 0.0001$; P2, P5 $p < 0.01$) and a numerical increase at P6 ($p = 0.06$).

In dioestrus, **prostaglandin $F_{2\alpha}$** caused an increase in intrauterine pressure at P1 to P4 (P1, P2, P4 $p < 0.0001$; P3 $p < 0.01$) and a decrease from 23.7 ± 54.98 mmHg to 8.6 ± 24.97 mmHg at P5 ($p < 0.001$). At P6 there was a non-significant numerical decrease in pressure. In oestrus, $PGF_{2\alpha}$ caused a significant increase in pressure from 7.3 ± 13.64 mmHg to 16.6 ± 17.41 mmHg at P1 ($p < 0.0001$).

Duration of effect of the experimental drugs

In dioestrus, **carbetocin** caused a significant increase in intrauterine pressure for 60 minutes and in oestrus for 75 minutes (Table 2).

In dioestrus, **oxytocin** did not cause an increase in pressure; in the time periods 5–8 (starting at minute 46 after medication) the pressure was significantly reduced compared to baseline. In oestrus, oxytocin caused a significant increase in pressure at the time period 3 and a numerical increase at time periods 2 and 4 (both $p = 0.06$; Table 2).

In dioestrus, **prostaglandin E_2** caused a significant increase in intrauterine pressure for 45 minutes (time periods 2–4). In time period 5, the pressure did not differ from baseline and in time periods 6–8, the pressure was significantly lower than baseline (all $p < 0.0001$). In oestrus, prostaglandin E_2 caused a significant increase in pressure for 60 minutes (Table 2).

In dioestrus and oestrus, **prostaglandin $F_{2\alpha}$** caused a significant increase in intrauterine pressure for 30 minutes (both $p < 0.01$). In dioestrus, this was followed by baseline pressure during time period 4 and decreased pressure during time periods 5–8 ($p < 0.05$). In oestrus, the pressure exceeded base line pressure again in time period 7 ($p \leq 0.05$; Table 2).

Discussion

Cycle-stage related pressure differences

Transport of semen from the cervix to the uterotubal junction constitutes a critical function of the bovine uterus during oestrus and thus, increased myometrial tone at this time of the oestrous cycle is expected, and was indeed confirmed in this study. The intrauterine pressure was greater in oestrus than in dioestus both before and after medication with four uterotonic drugs. This was in agreement with results of other studies in cows that showed greater intrauterine pressure amplitude during oestrus (2, 9, 18, 27, 28, 32). One of these studies also documented a greater area under the pressure curve, which was shown in vitro to be attributable mainly to the activity of the circular myometrial layer (18). During oestrus, the bovine uterus also had increased baseline pressure (28), longer duration of contractility (8, 14, 31) and increased uterine activity expressed in Montevideo units (32).

The reason for the differences in myometrial activity is the dominance of progesterone during dioestrus and the dominance of estradiol during oestrus (2, 36). Progesterone correlates

negatively with myometrial activity and estradiol correlates positively, which was demonstrated by exogenous administration of these hormones to ovariectomised cows (2). Estrogens increase the uterine endometrial and myometrial oxytocin receptor concentration in oestrous cows, whereas progesterone decreases the concentration (5, 13, 22).

Segmental pressure differences

This study revealed several significant pressure differences among various uterine segments both before and after medication with uterotonic drugs, which was in contrast to other studies that used pressure transducers with multiple miniature pressure sensors, but failed to record significant intersegmental differences (15–17, 19, 28). Segmental uterine pressure differences were measured in one older study (36) and isolated myometrial specimens from near the tip of the uterine horn had greater amplitudes and areas under the contractility curve than myometrial specimens from near the uterine body (23). This was in agreement with our findings that the lowest intrauterine pressures in dioestus were measured in the region of the uterine horn near the uterine body at P3 and P4. This finding combined with the greater pressure measured in the uterine body and cervix may be the reason for less efficient evacuation of pathological uterine content from the uterus during dioestrus compared with oestrus. During oestrus, maximum pressures were recorded in the middle of the horn at P2 and P3, intermediate pressures at P1 and P4 and the lowest pressure in the uterine body at P5. A similar decrease in intrauterine pressure from the horn towards the cervix was also recorded in an in-vitro study of bovine myometrial contractility (23). Such a pressure gradient should facilitate the transport of pathological uterine content towards the cervix. In contrast, a greater pressure was measured near the cervix than in the horn, which would impede uterine evacuation (36).

Cervical pressure was considerably higher in oestrus than in dioestrus (10.4 ± 17.91 mmHg vs. 3.6 ± 19.40 mmHg). This appears counterintuitive because of the general expectations that the cervix facilitates the passage of semen in oestrus. However, compared with the uterine horn pressure, cervical pressure is lower in oestrus and higher in dioestrus. It appears therefore that sperm transport through the cervix is not governed solely by the absolute cervical pressure but by the pressure gradient in the entire uterus.

All four tested drugs caused an increase in uterine horn pressure during dioestrus and oestrus. During dioestrus, cervical pressure decreased after oxytocin and $PGF_{2\alpha}$ but did not change significantly after carbetocin and PGE_2. During oestrus, none of the drugs affected cervical pressure significantly, which was surprising and contradictory with respect to PGE_2. This hormone caused cervical relaxation in vitro in isolated specimens from the uterus of pregnant women and late-term guinea pigs (10, 11, 31). In cattle, PGE_2 shortens the duration of labour and has been used for the removal of mummified foetuses (20, 21).

Duration of effect of uterotonic drugs

The duration of effect of all four drugs was longer in oestrus than in dioestrus. **Carbetocin** had the longest effect both in oestrus (75 minutes) and dioestrus (60 minutes) and was the only drug that did not result in a decrease in intrauterine pressure below baseline. The effect of carbetocin has not been evaluated during the oestrous cycle of cattle, but it increased intrauterine pressure for 1 hour, 14–16 hours postpartum (6). The duration of effect of carbetocin in the mare is longer compared with oxytocin (1). In myometrial specimens from late-term women, carbetocin and ocytocin caused similar increases in contractility (26), whereas in rats, carbetocin had a longer effect in similar in-vitro experiments (4). Carbetocin has a longer duration of effect because of its considerably longer half-life (up to 6 hours) compared with oxytocin (1–8 minutes) (5).

Oxytocin had the shortest duration of effect; a significant increase in intrauterine pressure only occurred during the first 15 minutes after administration in oestrus. In dioestrus, the pressure did not increase and instead decreased significantly below baseline after 45 minutes until the end of the measuring period. Similarly, intrauterine pressure was elevated for 1–3 minutes (14) or up to 27 minutes after intravenous administration (27). Oxytocin caused

contractions during oestrus but not on day 11 of the cycle in cows (12) or increased uterine pressure for 15–20 minutes in oestrus and dioestrus (30). The short duration of effect of oxytocin is explained by its short half-life (5). It is possible that in the present study, short-term pressure increases may have occurred during dioestrus, but did not affect the mean of the 15-minute period of measurement.

PGE_2 had the second longest duration of effect in oestrus (60 minutes) as well as in dioestrus (45 minutes). In dioestrus, the period of uterotonicity was followed 15 minutes later by pressures below baseline. In another study, PGE_2 caused a 20-minute increase in uterine tonicity at different cycle stages which however was not followed by a decrease below baseline in dioestrus, possibly because drug administration was repeated after 30 minutes (29). A significant increase in intrauterine pressure lasting 45 minutes also occurred after the intravenous administration of 1.25 mg, 2.5 mg and 5 mg PGE_2 to dioestrous cows (17, 19).

The 30-minute increase in intrauterine pressure after **$PGF_{2\alpha}$** in oestrous and dioestrous cows was shorter than after carbetocin and PGE_2. In oestrus, this was followed by a transient pressure decrease, after which the pressure increased again. In dioestrus, this was followed by a pressure decrease that lasted until the end of the measuring period. Other authors observed a pressure increase in dioestrus but not in oestrus (9, 34) or increases at both cycle stages of up to 17 minutes and 20 minutes, respectively (27, 29). A significant 30-minute pressure increase similar to our study was seen in dioestrous cows, but it was followed by a 90-minute plateau, rather than a decrease below baseline (15). The reason for this discrepancy is not known. Until now a comparable pressure decrease was only described in one study in cows during oestrus and dioestrus and occurred within 30 minutes after administration of cloprostenol (29). In our study drops in intrauterine pressure below baseline and the resulting negative intrauterine pressures were also seen after PGE_2 and oxytocin in dioestrus. Based on these finding it may questionable whether the use of these drugs would be beneficial during dioestrus.

The timing of calibration of a pressure catheter is critical for the recording of negative pressure. It is plausible that the relaxed uterus adopts a negative pressure after adaption to the normal negative pressure in the abdomen, provided that the cervix and vagina form a sufficient seal to prevent equalisation of pressure. In the present study, calibration of the transducer was achieved before insertion into the uterus and thus, baseline pressure corresponded to barometric pressure. This allowed us to record actual absolute negative pressures relative to the relatively stable barometric pressure. In contrast, when calibration was achieved some time after insertion of the transducer into the uterus, baseline pressure corresponded to intrauterine pressure and pressure changes were measured relative to the initial intrauterine pressure (15). In that study, negative intrauterine pressures did not occur. Direct comparison of intrauterine pressures across various studies was difficult because most authors did not specify the time of calibration.

From a clinical point of view these findings are crucial, because the therapeutic effect of an uterotonic drug is not only based on the ability to increase uterine pressure but also to prevent a pressure drop below baseline. Based on our 2-hour measurement period, in dioestrus PGE_2 caused pressure increases and decreases of equal length. $PGF_{2\alpha}$ caused a longer period of pressure decrease than increase, and the effect of oxytocin was limited to a pressure decrease when given in dioestrus. It is therefore possible that these drugs are contraindicated in dioestrus when an increase in intrauterine pressure is the goal. Except for oxytocin, the drugs used in this study can be expected to cause a transient increase in intrauterine pressure when given for the purpose of evacuation of pathological material; however, the relaxation that follows the pressure increase could contribute to further pooling of retained material. Studies involving cows with metritis and using longer measuring times are needed to investigate the therapeutic efficacy of uterotonic drugs.

Carbetocin caused the longest-lasting increase in intrauterine pressure, and in dioestrus a drop in pressure below baseline did not follow the increase. Carbetocin is therefore the best option when the goal of treatment is prolonged uterine contraction. $PGF_{2\alpha}$ causes a short-lived pressure increase in dioestrus, but the oestrus period that follows luteolysis is associated with

increased uterine tone. In clinical field studies the direct effect of $PGF_{2\alpha}$ on the uterine pressure to cure endometritis is controversial (25, 33). However the induction of oestrus through luteolysis seems to be beneficial in cows with endometritis after the first month post partum (33). Both strategies benefit the evacuation of the uterus and might also complement one another. If the therapeutic target is an immediate increase of the intrauterine pressure, carbetocin provokes the best direct uterotonic effect in both cycle stages. The effects of the four uterotonic drugs on the various uterine segments were similar. The selectively different effects of PGE_2 on the cervix and the remaining parts of the uterus which are typical of the peripartum period of different various species (10, 11, 21, 31) could not be confirmed in dioestrus and oestrus.

Conclusion for practice

■■■ Compared to the other three uterotonic drugs Carbetocin may be the best option when the aim of treatment is an immediate and prolonged uterine contraction. During estrus $PGF_{2\alpha}$, PGE_2 and Oxytocin caused a shorter pressure increase, which may useful evacuating pathological content from the uterus. However, the subsequent pressure drop below baseline could jeopardise this. In contrast to Carbetocin, $PGF_{2\alpha}$ and PGE_2 oxytocin caused no measurable pressure increase during diestrus.

Competing interests
The authors declare that they have no competing interests.

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Fig. 1 Microchip precision pressure catheter in the reproductive tract of a cow (pathological preparation). The left uterine horn and part of the vagina, but not the uterine body and cervix, have been opened. The rubber band sutured to the perineum and tied to the top ring of the catheter is not shown. (A = incised left uterine horn, B = cervix, C = partially opened vagina, D = tubing used to fix the pressure catheter in place, P1–P4 = measuring points of the pressure catheter in the uterine horn (P5 in the uterine body and P6 in the cervix are not visible).

Abb. 1 Mikrochip-Präzisionsdruckmesskatheter im Reproduktionstrakt einer Kuh (Sektionspräparat). Das linke Uterushorn und Teile der Vagina wurden eröffnet, jedoch nicht der Uteruskörper und die Zervix. Das am Perineum fixierte und zum Hülsenring gespannte Gummiband ist nicht dargestellt. (A = eröffnetes linkes Uterushorn, B = Zervix, C = teileröffnete Vagina, D = Druckkatheter in Fixationshilfen, P1–P4 = Messpunkte des Druckkatheters im Uterushorn (nicht sichtbar sind P5 im Uteruskörper und P6 in der Zervix).

Fig. 2 Overall intrauterine pressure during dioestrus (green) and oestrus (orange) in eight time periods for all drugs combined. (↓ = drug administration; * $p < 0.001$).

Abb. 2 Vergleich der Uterusdrücke im Diöstrus (orange) und Östrus (grün) anhand der acht Zeitabschnitte unabhängig vom verwendeten Medikament und den Messpunkten im Uterus. (↓ = Zeitpunkt der Medikation; * $p < 0{,}001$).

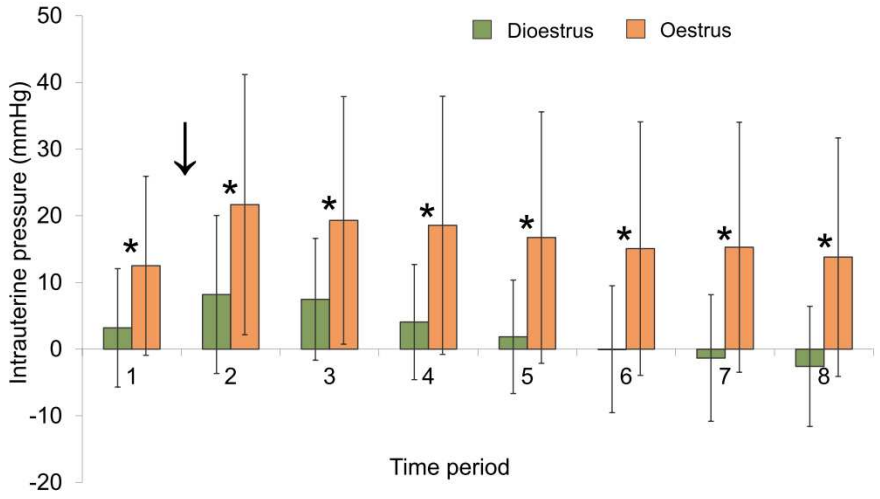

Fig. 3 Intrauterine pressure at six measuring points before (time period 1 – baseline) and after medication (time period 2–8) in dioestrus for all drugs combined. (Columns with decreasing colour intensity correspond to measuring points 1–6; ↓ = drug administration)

Abb. 3 Vergleich der Uterusdrücke an den sechs Messpunkten vor (Zeitabschnitt 1) und nach Medikamentengabe (Zeitabschnitte 2–8) im Diöstrus unabhängig vom applizierten Medikament. (Säulen mit abnehmender Farbintensität entsprechen den Messpunkten 1–6. ↓ = Zeitpunkt der Medikation).

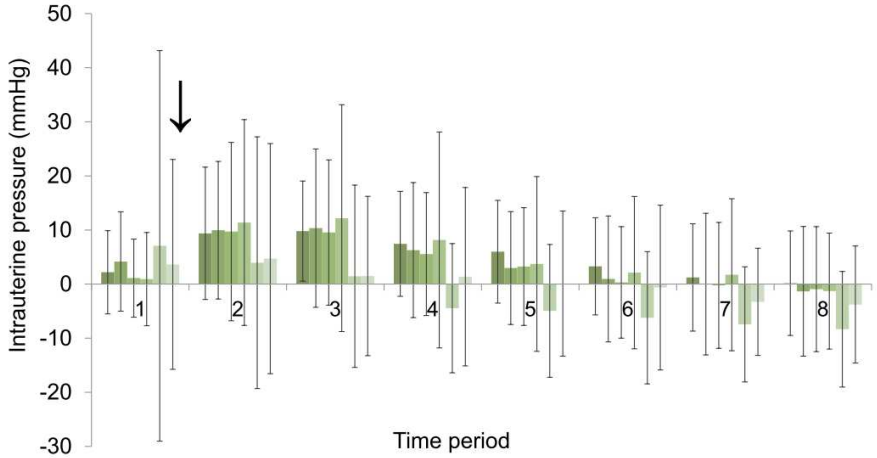

Fig. 4 Intrauterine pressure at six measuring points before (time period 1 – baseline) and after medication (time period 2–8) in oestrus for all drugs combined. (Columns with decreasing colour intensity correspond to measuring points 1–6; ↓ = drug administration).

Abb. 4 Vergleich der Uterusdrücke an den sechs Messpunkten vor (Zeitabschnitt 1) und nach Medikamentengabe (Zeitabschnitte 2–8) im Östrus unabhängig vom applizierten Medikament. (Säulen mit abnehmender Farbintensität entsprechen den Messpunkten 1–6. ↓ = Zeitpunkt der Medikation)

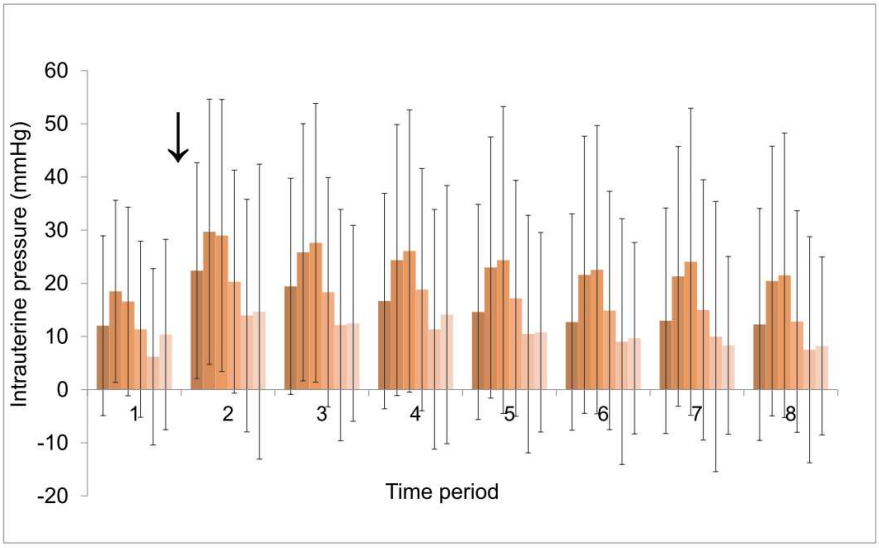

Fig. 5 Intrauterine pressure at six measuring points before (time period 1) and after medication (time period 3) in dioestrus for all drugs combined. (Columns with decreasing colour intensity correspond to measuring points 1–6; * p ≤ 0.05)

Abb. 5 Vergleich der Uterusdrücke an den sechs Messpunkten zwischen Zeitabschnitt 1 vor der Medikation und Zeitabschnitt 3 nach der Medikation im Diöstrus unabhängig vom applizierten Medikament. (Säulen mit abnehmender Farbintensität entsprechen den Messpunkten 1–6; * p ≤ 0,05).

Fig. 6 Intrauterine pressure at six measuring points before (time period 1) and after medication (time period 3) in oestrus for all drugs combined. (Columns with decreasing colour intensity correspond to measuring points 1–6; * p ≤ 0.05)

Abb. 6 Vergleich der Uterusdrücke an den sechs Messpunkten zwischen Zeitabschnitt 1 vor der Medikation und Zeitabschnitt 3 nach der Medikation im Östrus unabhängig vom applizierten Medikament. (Säulen mit abnehmender Farbintensität entsprechen den Messpunkten 1–6; * p ≤ 0,05).

Table 1 Intrauterine pressure (mmHg) at six measuring points before (time period 1) and after medication (time period 2–8) for all drugs combined. (Superscript numbers indicate measuring points with significantly different pressure; $p < 0.05$).

Tab. 1 Uterusdrücke (mmHg) an den sechs Messpunkten vor (Zeitabschnitt 1) und nach der Applikation der Medikamente (Zeitabschnitt 2–8). (Hochgestellte Ziffern markieren Messpunkte mit signifikant differierenden Druckwerten; $p < 0,05$).

Table 2 Intrauterine pressure (mmHg) before (time period 1) and after medication (time periods 2–8). (Superscript numbers indicate time periods with significantly different pressure. Letters in bold indicate a significant pressure increase, letters in italic indicate a significant pressure decrease compared with baseline pressure ($p \leq 0.05$).

Tab. 2 Uterusdrücke (mmHg) vor (Zeitabschnitt 1) und nach der Applikation der Medikamente (Zeitabschnitt 2– 8). (Hochgestellte Ziffern markieren Messpunkte mit signifikant differierenden Druckwerten. Signifikante Druckanstiege sind durch Fettdruck hervorgehoben, signifikante Druckabfälle durch Kursivdruck; $p \leq 0,05$).

Table 1

		P1	P2	P3	P4	P5	P6
Time period 1	Dioestrus	2.2 ± 7.69^{2345}	4.2 ± 9.19^{134}	1.1 ± 7.22^{1256}	0.9 ± 8.63^{1256}	7.1 ± 36.10^{134}	3.6 ± 19.40^{34}
	Oestrus	12.0 ± 16.90^{235}	18.5 ± 17.12^{1456}	16.6 ± 17.73^{1456}	11.4 ± 16.57^{235}	6.2 ± 16.59^{12346}	10.4 ± 17.91^{235}
Time period 2	Dioestrus	9.4 ± 12.24^{56}	10.0 ± 12.74^{56}	9.7 ± 16.49^{56}	11.4 ± 19.04^{56}	4.0 ± 23.28^{1234}	4.7 ± 21.27^{1234}
	Oestrus	22.4 ± 20.29^{2356}	29.7 ± 24.92^{1456}	29.0 ± 25.58^{1456}	20.3 ± 20.98^{356}	13.9 ± 21.88^{1234}	14.7 ± 27.75^{1234}
Time period 3	Dioestrus	9.8 ± 9.25^{456}	10.4 ± 14.64^{56}	9.5 ± 13.44^{456}	12.2 ± 20.97^{1356}	1.5 ± 16.87^{1234}	1.5 ± 14.74^{1234}
	Oestrus	19.4 ± 20.32^{2356}	25.8 ± 24.19^{1456}	27.6 ± 26.22^{1456}	18.3 ± 21.57^{2356}	12.1 ± 21.75^{1234}	12.5 ± 18.42^{1234}
Time period 4	Dioestrus	7.4 ± 9.70^{356}	6.3 ± 12.48^{56}	5.6 ± 11.36^{1456}	8.2 ± 19.96^{356}	-4.4 ± 11.95^{12346}	1.4 ± 16.49^{12345}
	Oestrus	16.7 ± 20.26^{235}	24.4 ± 25.49^{1456}	26.1 ± 26.53^{1456}	18.8 ± 22.77^{2356}	11.3 ± 22.54^{1234}	14.1 ± 24.28^{234}
Time period 5	Dioestrus	6.0 ± 9.49^{23456}	3.0 ± 10.44^{156}	3.2 ± 10.88^{156}	3.7 ± 16.16^{156}	-5.0 ± 12.29^{12346}	0.1 ± 13.43^{12345}
	Oestrus	14.6 ± 20.22^{2356}	23.0 ± 24.56^{1456}	24.4 ± 28.86^{1456}	17.2 ± 22.18^{2356}	10.5 ± 22.33^{1234}	10.8 ± 18.77^{1234}
Time period 6	Dioestrus	3.3 ± 8.99^{2356}	1.0 ± 11.65^{15}	0.3 ± 10.31^{15}	2.1 ± 14.08^{56}	-6.2 ± 12.23^{12346}	-0.6 ± 15.23^{145}
	Oestrus	12.7 ± 20.34^{2356}	21.6 ± 26.07^{1456}	22.6 ± 27.11^{1456}	14.9 ± 22.42^{2356}	9.0 ± 23.12^{1234}	9.7 ± 17.99^{1234}
Time period 7	Dioestrus	1.2 ± 9.92^{56}	0.0 ± 13.10^{56}	-0.2 ± 11.64^{456}	1.7 ± 14.06^{356}	-7.4 ± 10.64^{12346}	-3.3 ± 9.92^{12345}
	Oestrus	13.0 ± 21.19^{236}	21.3 ± 24.43^{1456}	24.1 ± 28.87^{1456}	15.0 ± 24.49^{2356}	10.0 ± 25.40^{234}	8.3 ± 16.71^{1234}
Time period 8	Dioestrus	0.2 ± 9.66^{56}	-1.3 ± 12.00^{56}	-0.9 ± 11.56^{56}	-1.3 ± 10.73^{56}	-8.3 ± 10.69^{12346}	-3.8 ± 10.82^{12345}
	Oestrus	12.3 ± 21.82^{2356}	20.4 ± 25.36^{1456}	21.5 ± 26.76^{1456}	12.8 ± 20.82^{2356}	7.5 ± 21.25^{1234}	8.2 ± 16.73^{1234}

Table 2

Time	Carbetocin		Oxytocin		Prostaglandin E_2		Prostaglandin $F_{2\alpha}$	
period	Dioestrus	Oestrus	Dioestrus	Oestrus	Dioestrus	Oestrus	Dioestrus	Oestrus
1	2.8 ± 5.49^{2345}	14.6 ± 15.45^{23456}	5.3 ± 10.09^{5678}	14.5 ± 13.39^{3}	-1.0 ± 5.51^{234678}	9.5 ± 10.08^{2345}	5.6 ± 11.33^{235678}	11.3 ± 13.71^{237}
2	**6.9 ± 9.55^{1678}**	**20.3 ± 19.93^{1348}**	3.3 ± 8.47^{378}	17.2 ± 18.16	**13.7 ± 15.35^{1345678}**	**32.9 ± 19.45^{1345678}**	**8.8 ± 10.59^{145678}**	**15.6 ± 15.52^{1}**
3	**8.0 ± 6.83^{15678}**	**23.7 ± 21.21^{12678}**	6.6 ± 9.89^{25678}	**19.7 ± 17.56^{158}**	**6.4 ± 9.75^{1245678}**	**18.7 ± 16.58^{1245678}**	**8.9 ± 9.64^{145678}**	**15.1 ± 17.71^{1}**
4	**6.5 ± 8.10^{15678}**	**25.1 ± 20.13^{125678}**	4.2 ± 9.34^{578}	20.8 ± 18.32^{5678}	**1.6 ± 8.55^{1235678}**	**13.3 ± 18.80^{123}**	4.0 ± 7.92^{23678}	15.0 ± 17.86
5	**4.8 ± 8.21^{134678}**	**20.6 ± 21.22^{1478}**	0.7 ± 7.51^{1348}	16.4 ± 16.05^{34}	-0.9 ± 6.69^{234678}	**15.4 ± 19.75^{1236}**	2.7 ± 10.11^{12378}	14.5 ± 17.27
6	2.2 ± 9.25^{2345}	**18.7 ± 18.62^{1234}**	0.9 ± 8.81^{1378}	16.4 ± 15.06^{4}	-4.5 ± 8.05^{1234578}	10.4 ± 20.99^{235}	1.9 ± 10.20^{12378}	15.1 ± 19.63
7	2.7 ± 7.55^{2345}	17.6 ± 18.05^{345}	0.4 ± 10.70^{123468}	15.3 ± 17.70^{4}	-6.6 ± 7.05^{123456}	13.1 ± 19.34^{23}	-0.9 ± 9.87^{123456}	**15.1 ± 19.82^{1}**
8	0.8 ± 7.58^{2345}	15.4 ± 19.55^{2345}	-1.7 ± 8.28^{1234567}	14.7 ± 14.33^{34}	-6.3 ± 7.87^{123456}	12.2 ± 20.49^{23}	-2.4 ± 10.48^{123456}	12.8 ± 15.64

Vorschlag zur Neugliederung

Material and methods

Animals

Six multiparous Swiss Braunvieh cows that were at least 8 weeks in milk and between 5 and 16 years old from a university-owned research farm were used. The cows had to be assessed healthy in the clinical and gynaecological examinations before they were included in the experiment.

Study design

Each of the six cows underwent one pressure measurement with each of four experimental drugs during oestrus and during dioestrus for a total of 48 measurements.

Determination of cycle stage

To determine the cycle stage, the ovaries of the cows were examined transrectally once daily using B-mode sonography until ovulation was detected, which was defined as the first day that a follicle was no longer visible on the sonograms (day 0). After ovulation, the daily examinations were continued to verify the cycle stage.

A corpus luteum with a diameter ≥ 23 mm combined with a plasma progesterone concentration > 1 ng/ml was defined as dioestrus (7, 8). A corpus luteum with a diameter < 23 mm combined with a plasma progesterone concentration ≤ 1 ng/ml and one or several follicles with a diameter ≥ 12 mm was defined as oestrus.

For determination of plasma progesterone concentration, 10 ml blood was collected from a jugular vein into a lithium heparin tube (Sarstedt, Nümbrecht, Germany) and centrifuged. The harvested plasma was stored at –18 °C until analysis. Progesterone was measured at the Endocrinology Laboratory at the Clinic for Obstetrics, Gynaecology and Andrology for Large and Small Animals and Ambulatory Field Service, Justus-Liebig-University, Giessen, Germany using a radioimmunoassay (24).

Application of uterotonic drugs

Experimental drugs included carbetocin (LongActon®, Vital, Switzerland), oxytocin (Physovetin®, Streuli Pharma, Switzerland), prostaglandin E_2 (Dinoproston, Myoton E_2®, Graeub, Switzerland) and prostaglandin $F_{2\alpha}$ (Dinoprost, Dinolytic®, Zoetis, Switzerland). The dosages and routes of administration were chosen according to the manufacturers' recommendations and were as follows: 30 IU for oxytocin, intramuscularly (i. m.); 0.35 mg for carbetocin, i. m.; 25 mg for prostaglandin $F_{2\alpha}$, i. m.; and 2.5 mg for prostaglandin E_2 2.5 mg, intravenously into a jugular vein. The triceps muscle of the forearm was used for intramuscular injections.

Oxytocin or carbetocin were tested on day 10 and PGE_2 or $PGF_{2\alpha}$ after a wash-out period of 48 hours (day 12) of the same cycle. Oxytocin or carbetocin was then tested on the first day of the next oestrus, and PGE_2 or $PGF_{2\alpha}$ after a wash-out period of 24 hours to ensure that both measurements were carried out during the same oestrus. The same course of measurements was done starting with the second peptide hormone (oxytocin or carbetocin) and the second prostaglandin (PGE_2 or $PGF_{2\alpha}$).

Pressure catheter and fixation

The pressure catheter was a Gastrobar® microchip precision pressure catheter with six semi-conductor pressure sensors (microtransducers) custom-made for this study (Raumedic, Münchberg, Germany). The probe had an average diameter of 3.5 mm and the first pressure sensor (P1) was 2 cm from the tip of the probe (Fig. 1). The remaining pressure sensors (P2 to P6) were located at 5-cm intervals so that the sixth sensor was 27 cm from the tip of the probe.

The microchip precision pressure catheter was introduced into a uterine horn via a sheath (modified Detlef biopsy instrument reaching from the handle to the external cervical os; Fig. 1). After placement of the pressure catheter (A, green catheter visible in the opened uterine horn and at the caudal end of the sheath) a piece of plastic tubing (D, custom-made from the sheath of a swabbing instrument) with the same length as the sheath and with a slit along its entire length was clipped onto the catheter piece by piece and slid towards the cervix. The caudal end of the tubing rested against the two rings at the caudal end of the sheath, which was attached to the perineal tissue with an elastic band (not shown in Fig. 1). The tubing served to fix the pressure catheter in place. A 20-mm bulb attached to the tip of the stealth was lodged against the external cervical os by force of the elastic perineal attachment and kept the tube and catheter from entering the cervix. The premeasured position of the 77-cm mark of the pressure catheter at the caudal end of the catheter ensured that the most caudally located measuring point P6 was located 4 cm cranial to the bulb-end of the plastic tubing in the cervix. The pressure data were transmitted to an attached measuring unit (Ellipse, Andromeda, Taufkirchen/Potzham, Germany) and from there to a laptop. The software AUDACT (Andromeda) was used to store and analyse the data.

Pressure measurement procedure and data processing

The pressure catheter was calibrated to barometric pressure and set to 0 before intrauterine placement. The cows were allowed 20 minutes to adapt to the catheter. At the beginning of every measurement (four in dioestrus and four in oestrus per cow), recordings of the physiological uterine pressure were carried out for 15 minutes (baseline measurement). The test drug was then given and the pressure recorded for another 105 minutes. Measuring artefacts defined as impacts on the pressure sensors that were not related to changes in myometrial tone such as urination, defecation, coughing, vocalisation or straining to expel air from the vagina were noted.

Heart and respiratory rates and possible side effects were recorded before the start of the experiment, 10 minutes after the start of pressure recordings and 5, 30, 60 and 120 minutes after administration of the test drug.

Each measuring point of the pressure catheter yielded data sets with 20 pressure readings per second. These data sets were transferred to an Excel spread sheet (Microsoft, Wallisellen, Switzerland) and means were calculated for a 15-minute period (minutes 15–29, referred to as baseline) before and seven 15-minute periods (minutes 31–45, 46–60, 61–75 etc.) after drug administration. The readings from the six measuring points were used for comparison of the pressure in different uterine segments, and the means of all six measuring points were used for comparison of the overall pressure. In case of artefacts, a software program (AUDACT, Andromeda) was used to delimit and eliminate the affected recordings, and the means were calculated for the remaining data of the respective 15-minute period.

Endpoints

Oestrous cycle-related changes in overall uterine pressure were analysed using the means of the eight time periods during oestrus and dioestrus for all drugs combined.

Differences in pressure between individual measuring points were analysed for all eight time periods during dioestrus and oestrus for all drugs combined. Differences in pressure between individual measuring points were analysed during oestrus and dioestrus for each drug by comparing time periods 1 and 3. Time period 3 (i. e., the second after medication) was associated with the greatest pressure increase for all pressure points.

The duration of effect of each drug was determined during dioestrus and oestrus by comparing the mean overall pressure during time period 1 with the pressures during time periods 2 to 8. This allowed determination of the duration of effect for each drug with an accuracy of 15 minutes.

Statistics

The mean uterine pressures recorded at the eight measuring periods were analysed using StatView Version 5.0 (SAS Institute, Cary, USA). The Shapiro-Wilk test was employed to test the data for normal distribution. Because all data were normally distributed, they were reported as mean ± standard deviation. Including the individual differences in the statistical models differences in pressure between time periods were analysed for both cycle stages separately or combined for each drug using a paired t-test.

Student's t-test for paired samples was used to analyse differences in mean overall pressure at all time periods between oestrus and dioestrus for each drug separately and for all drugs combined, to analyse differences in mean pressure at the individual measuring points and in each time period for all drugs and both cycle stages combined and to analyse differences in mean pressure recorded at the different measuring points between time periods 1 and 3. A p-value ≤ 0.05 was considered significant.

This study was approved by the Committee for the Permission of Animal Experimentation of the Canton of Zurich (04/2007).

Vorschlag zur Neugestaltung von Abbildung 3 und 4. Die ausgewählten Farben korrespondieren mit den in der Zeitschrift verwendeten Farben. So erscheinen die Abbildungen nicht zu bunt. Die Unschärfe der Abb. hier resultiert daraus, dass es sich um einen eingefügten Screenshot handelt.

Fig. 3 Intrauterine pressure at six measuring points before (time period 1 – baseline) and after medication (time period 2–8) for all drugs combined in dioestrus (a) and oestrus (b). (Columns with decreasing colour intensity correspond to measuring points 1–6; ↓ = drug administration).

Abb. 3 Vergleich der Uterusdrücke an den sechs Messpunkten vor (Zeitabschnitt 1) und nach Medikamentengabe (Zeitabschnitte 2–8) im Diöstrus (a) und Östrus (b) unabhängig vom applizierten Medikament. (Säulen mit abnehmender Farbintensität entsprechen den Messpunkten 1–6. ↓ = Zeitpunkt der Medikation).